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A transitional B cell cytokine biomarker for risk stratifying renal transplant patients with borderline rejection

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Abstract
Borderline allograft rejection can promote acute rejection and graft loss in some, but not all patients. In this issue, Cherukuri et al. [1] use a novel test based on peripheral blood transitional T1 B (T1B) cells producing IL-10 & TNFα which identifies patients at high-risk for poor outcomes. The potential mechanisms by which T1B cells might modulate alloreactivity need exploration, but following appropriate validation, this biomarker could risk stratify patients in need of early intervention.

Main text
Borderline change (BL), the term used to describe mild to moderate interstitial infiltration accompanied by foci of tubulitis, has been included in the Banff classification of allograft rejection since its inception in 1991. Early protocol biopsy series reported that BL was associated with poorer renal function compared to kidneys demonstrating normal histology. If discovered on for-cause biopsies taken for graft dysfunction, a high proportion with BL go on to develop acute cellular rejection (ACR), progressive functional impairment and graft loss. In contrast, if BL was discovered on surveillance biopsies, the risk of subsequent clinical ACR, graft dysfunction and graft loss is lower, and inflammation can spontaneously resolve in >60% of cases [2]. Identifying those cases of BL at highest risk of pathological progression is therefore an important clinical goal.

Evidence is building that sophisticated analysis of the mismatched HLA epitopes available for T cell recognition may inform risk stratification for poor clinical outcomes [3]. RNA transcriptome analysis of biopsy tissue to identify molecular signals of inflammation associated with immune infiltrate has been successfully validated. This approach is limited by the invasive nature of procuring the biopsy as well as the potential for sampling error inherent to histological or molecular analyses of biopsy tissues. The GoCar study overcame this by identifying and validating a 17 gene signature in peripheral blood that strongly correlated with the diagnosis of BL in 3-month protocol biopsies that developed into clinically evident ACR, progressive graft dysfunction and graft loss [4]. This signature is currently undergoing larger scale clinical validation.

In this edition, Cherukuri et al. [1] perform a retrospective observational cohort study evaluating 851 patients (representing 70% of patients transplanted between 2013-2018 in their centre) who had surveillance or for-cause biopsies in the first 4 months after transplantation. A total of 217 (25%) had BL from either surveillance (171/217; 79%) or for-cause (46/217; 21%) biopsies, while 387 (45%) had no significant inflammation (NI). 80% of this cohort also had later biopsies at 5-12 months post-transplant. The authors utilised 2019 Banff criteria, which defines BL as foci of tubulitis (t1, t2, or t3) with mild interstitial inflammation (i1), or mild (t1) tubulitis with moderate-severe interstitial inflammation (i2 or i3) without arteritis (v = 0) [5]. Of note, approximately 40% received treatment for the BL. Interstitial fibrosis / tubular atrophy (IFTA) or donor specific antibody (DSA) did not preclude inclusion but these did not significantly influence outcomes. Concurrently, 53 of the 217 (24%) BL

patients and 105 of the 387 (27%) with NI consented to peripheral blood mononuclear cell (PBMC) cryopreservation.

The authors confirmed that, compared to NI, BL in early biopsies was associated with increased risk of ACR (occurred in 40% with BL vs. 20% with NI), worse IFTA in the late biopsies, and an increased risk of graft loss after 7 years. Notably, these outcomes were no different in the 40% of patients who were treated for BL. In trying to identify factors associated with poorer long-term outcomes, they used T-distributed stochastic Neighbourhood Embedding (tSNE), an unsupervised non-linear of dimensionality reduction algorithm to cluster patients with BL into 5 distinct neighbourhoods based on 41 early histological and clinical parameters. These 41 independent variables included donor and recipient characteristics, HLA mismatches and DSA, early histology scoring and early transplant events (including EBV and CMV mismatches, cold ischemia time (CIT), delayed graft function, treatment of BL and maintenance steroids at 1 y). While these were well-described risk factors for graft survival, they concluded that tSNE provided early risk stratification for BL, but its performance was inferior to late risk stratification of patients with poor 7-year graft survival. In contrast, when tSNE was performed in NI patients, categorization based on CIT, kidney donor profile index and donor type that risk-stratified BL patients, did not differentiate the clinical course of NI patients in terms of late rejection, IFTA or death-censored graft survival.

These authors then asked whether their previously published observations on the utility of examining IL-10 and TNFα production by T1B cells after polyclonal stimulation, would be able to risk stratify BL outcomes [6,7]. They examined this variable in the subset of patients (~25%: 53 BL, 105) NI who had consented to PBMC cryopreservation. PBMC were stimulated with NIH-3T3 cells stably expressing CD40L, CpG-ODN-2006 for 19 h, then phorbol 12-myristate 13-acetate and ionomycin was added for 5 h, and the frequency B cells producing IL-10 vs TNFα was determined. Furthermore, the B cells were further divided into T1B (CD24+++CD38+++) T2B (CD24++CD38++) naïve (CD24+CD38+) and memory (CD24+++CD38-) cells. By employing a threshold ratio of 1.3, based on the frequency of B cells that were producing IL-10 vs TNFα, they showed that the IL-10/TNFα ratio in total B, TrB, and T1B cells in BL patients that developed late AR was significantly lower than NI patients or BL patients that did not develop late AR. Furthermore, NI patients and BL patients that did not develop late AR had comparable cytokine ratios. Of the B cell subsets, the IL10/TNFα ratio in T1B most accurately distinguished BL patients that did vs did not develop late AR. This had a receiver operating characteristic area under the curve (ROC-AUC) of 0.87 (95% CI, 0.72 to 1.00; $p<0.0001$) for predicting subsequent late AR.

With a cut-off value of 1.3 for the IL-10/TNFα ratio, the sensitivity for the prediction of subsequent late AR in BL patients was 80%, specificity 94%, and with a negative predictive value (NPV) of 91% and positive predictive value (PPV) of 86%. At this threshold, 28% of BL patients were high risk and 86% developed late AR and had markedly worse 7-year death-censored graft survival compared to low-risk BL patients (death-censored graft survival: 55% vs. 92%). In contrast, 72% were low risk, and only 9% developed late AR, while 55% resolved and 36% remained BL. Thus, the T1B cytokine ratio measured 3 months post-transplantation had high sensitivity, specificity, positive and negative predictive values in both BL and NI groups to ascertain worse clinical outcomes (Figure 1). The authors conclude that the evaluation of T1B IL-10/TNFα ratio provides a non-invasive test to risk stratify clinically relevant BL as early as 3 months post-transplantation.

The clinical implication of these observations is that this biomarker should prompt early surveillance biopsies to identify the patients with BL and poor prognosis under standard-of-care therapies. Indeed, the authors discuss that conventional treatments such as methylprednisolone or augmented immunosuppression appeared not to influence outcomes in the 40% treated in this cohort. They therefore speculated that novel therapies such as TNF blockade might be a more rational approach [8].

Most importantly, the authors acknowledge that their findings require further validation in other cohorts before generalisability can be assumed. In addition, a comparison or integration with other risk predictive biomarkers, some of which are mentioned above, is needed.

Observations from this study raise a number of mechanistic questions. Specifically, why is the ratio of T1B cell cytokine an effective biomarker for risk stratification? One potential explanation is that these T1B cells enter secondary lymphoid organs and modulate alloreactive T and B cell priming as a result of their production of immunomodulatory IL-10, thus functioning as regulatory B cells [9]. An alternative interpretation is based on the classical definition that T1B cells have recently emerged from the bone marrow and are en route to secondary lymphoid organs, where a fraction of T2B cells escape negative selection and develop into mature B cells. Thus, the ratios of T1B cells producing IL-10 and TNFa may reflect polymorphisms that control the propensity of all recipient immune cells to produce these cytokines. Under this hypothesis, T1B would most closely reflect the recipient's genetic propensity, as the more mature B cell repertoire may be additionally shaped by exposure to cytokines and other B cell activating signals upon passage through secondary lymphoid organs. Examination of whether pre-transplant IL-10/TNFα ratios in T1B cells are also predictive of graft outcomes as ratios determined post-transplantation may address these possibilities.

The pre-transplant identification of at-risk recipients for poor outcomes, as well as the identification of at-risk BL recipients, allows for pre-emptive tailoring of immunosuppression to prevent the development of alloreactive immune responses. The ability to implement individualized immunosuppressive therapy will spare low-risk patients from unnecessary side-effects of over immunosuppression without benefits of better graft outcome, and ensure that high risk patients are adequately immunosuppressed.

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Figure Legend

A: PBMC taken 2-4 months post-transplantation from 53/217 patients with BL and 105/387 with NI were stimulated in vitro with CD40L and a TLR9 agonist, followed by incubation with PMA, ionomycin and brefeldin, and intracellular staining for IL-10 and TNFa. The ratio of frequency of CD38++CD24++ T1B cells producing IL-10 vs TNFα was significantly lower in the BL group that had those who experienced acute rejection (n=15) or no acute rejection (n=33) in later biopsies. The threshold ratio of 1.3 was chosen to split patients into those at low (ratio >1.3) or high (ratio ≤1.3) risk of future AR. **B:** Amongst the high-risk BL group, 86% developed late AR and only 14% resolved. In contrast, only 9% of the low-risk group developed late AR, while 55% resolved and 36% remained BL. **C:** High-risk BL patients had markedly worse 7-year death-censored graft survival compared to low-risk BL patients (death-censored graft survival (55% vs. 92%). However, high risk patients with NI had statistically comparable graft survival to low-risk patients.